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Biodefense to Cancer Office-Meeting Transcirpt

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Biodefense to Cancer Offense
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From Biodefense to Cancer Offense
A Redacted Transcript of the Concluding Plenary Session

The following transcript has been significantly edited for relevance, grammar, diction, and sentence structure. Speakers are identified to aid follow-up communications and are not accountable for this narrative.

Introduction

On January 29, 2004, the UC Davis Cancer Center (UCDCC) and Lawrence Livermore National Laboratory (LLNL) held a workshop titled "Biodefense to Cancer Offense: An Exploration of Technology Transfer". The workshop was an outgrowth of a memorandum of understanding (MOU), signed by the UCDCC and LLNL in 2000. The MOU established an integrated cancer program, one of the program goals being to encourage collaborative activities leading to new technology discoveries in the control, detection, diagnosis, treatment, and management of cancer. This workshop was a focused effort to encourage those collaborations.

The workshop was an all-day event and by invitation only. Most of the approximately 100 attendees were researchers and clinicians, although senior management from both the UC Davis School of Medicine and the Director's Office at LLNL also attended. In an effort to broaden the potential for cross-discipline collaborations, workshop organizers invited participants from a wide range of disciplines and areas of expertise, including oncology, hematology, radiology, urology, pathology, surgery, veterinary medicine, biology, chemistry, physics, engineering, materials science, and biostatistics.

The workshop's focus was set early in the day when Dennis Matthews, co-chair of the workshop, opened the meeting by outlining the following goals for each attendee at the workshop.

- Learn about the needs of UCD/LLNL cancer researchers and clinicians.
- Learn about key technologies and expertise in LLNL's Counter Bioterrorism Program.
- Create a list of key medical needs vs. available or developable technologies.
- Create a list of hot topics for proposals.
- Form teams to generate proposals to NIH.
- Develop strategies for pursuing research projects that lead to seminal publications and scientific presentations.
- Create the means to pursue development of technologies into research or clinical devices.

The opening remarks were followed by of a series of 10 short presentations from UCDCC and LLNL. Each presentation lasted about 20 minutes with a few additional minutes devoted to Q&A. The goal of the Cancer Center presentations was to acquaint LLNL researchers with some of the current challenges in cancer diagnosis and therapy. Likewise, the goal of the LLNL presentations was to acquaint UCDCC researchers and clinicians with some of the anti-cancer potentials for existing and/or emerging biodefense technologies.

Titles of presentations, and the presenters, are listed below:

- "Clinical Cancer Research: Opportunities and Obstacles": *David R. Gandara, MD,*

Associate Director, Clinical Research, UCDCC

- "Cancer Cell Signals in Time and Space": *Ron Wisdom, MD, Leader, Tumor Biology in Animals, Division of Hematology Oncology, UCDCC*
- "Technological Opportunities in Cancer Surgery"*: Ralph de Vere White, MD, Director, UCDCC, and Chair, Department of Urology*
- "Technology Needs to Advance Molecular Studies in Clinical Cancer Research": *Paul Gumerlock, Ph.D., Leader, Prostate Cancer Program, UCDCC*
- "Approaches to Drug Discovery, Imaging Agents, and Proteomics": *Kit Lam, MD, Ph.D., Chief, Division of Hematology Oncology, Leader, Cancer Therapeutics Program, UCDCC*
- "LLNL Biodefense Technologies, Past, Present, Future": *Fred Milanovich, Ph.D., Program Leader Emeritus, Chemical and Biological National Security Program, LLNL*
- "Pathomics: Bio-markers of Infectious Disease"*: Ken Turteltaub, Ph.D., Division Leader, Biodefense Division, Biology and Biotechnology Research Program, LLNL*
- "High-throughput Gene Silencing and Cellular Imaging": *Allen Christian, Ph.D., Group Leader, Bioengineering, Biology and Biotechnology Research Program, LLNL*
- "Synthetic High Affinity Ligands (SHALs): From Detecting Bio-threat Agents to Targeting Tumor Cells"*: Rod Balhorn, Ph.D., Division Leader, Molecular Biophysics, Biology and Biotechnology Research Program, LLNL*
- "Instrumentation and Methods for Detection of Biodefense-Related Organisms": *Mary McBride, Ph.D., Group Leader, Medical Physics and Biophysics, Physics and Advanced Technologies, LLNL*

Following a lunch break, which included 15 poster sessions, the attendees split up into seven concurrent, pre-assigned breakout sessions. Workshop organizers seeded each session with (1) equal representation from both LLNL and the UCDCC, and (2) researchers with diverse backgrounds and interests. Sessions, however, had no assigned topics. Leaders for each session were pre-assigned, one from the Cancer Center and one from LLNL. The leaders' role was to keep the group focused on the goals of the workshop (as identified in the opening session) and to take notes of the discussions. The breakout sessions lasted approximately two hours.

Following the breakout sessions, attendees gathered together once more for a plenary session. During this session, the leaders from each of the breakouts reported back on the discussions that their group has focused on, both in response to the information provided during the morning presentations and to the workshop goals. Clearly, common themes ran through many of the break-out group discussions. Each group's report, however, reflected the interests and expertise of its members. The following is a redacted transcript of that plenary session.

Breakout Session 1

[Andy Wyrobek]
Our group discussed, in general, individualized medicine and improvements in pathology. We talked about the need for predictive markers for therapy response, for better staging, and for minimizing toxicity. We primarily talked about two biological systems, human studies and the mouse model. The technologies that we touched upon address small sample size problems, and the one we discussed was NMR technology. Fast super computers could be used for looking at images in multiple dimensions and storing information from the tissue images, etc.

We also discussed tissue repositories, mouse and human tissue preparation methods, the fact that quite often tissue-preparation methods are artificial; the stains that are used are an example of that. How can we really look at the tissue the way it was in the living organism?

Developing mathematical models and predictive algorithms for the different outcomes that I mentioned, tissue response, toxicity, and so on would be helpful.

We discussed discovery tools: large-scale discovery, gene transcript to some extent, mass spectrometry, cell imaging, 2-D gels, and variances of them. We also discussed Luminex protein analysis technology and how that could be scaled up to include more and more targets.

Needs

One was modeling the protein profiles of cancers at different stages so that we might be better able to diagnose a cancer. Modeling the tumor time course, aspects of tumor development, and seeing if the imaging can distinguish between a metastasis and primary tumors in terms of some aspects of the metabolism, and so on. We thought that might be done best in the mouse, and perhaps also in the human.

We spent quite a bit of time on digital pathology. Understanding the PET patterns in the human and the mouse, analyzing images other than just 2-dimensional images, going to 3-dimensional images.

Mathematical descriptors of the tumors and developing better algorithms with staging, something I mentioned earlier.

Computer presentation of tissue images or pathology images, linked to the diagnosis or the description by the pathologist, to be contrasted with what you currently get, a written descriptor unlinked to the image. The pathologist saw the image but the physician does not see the image, so having this together as a unit was an identified need.

Need to look at tissues without damaging the tissues. Need to predict whether a transplant will take before you actually undertake the operation, so it is a way of assessing the tissue health and the insides of the tissue. An example given was a kidney transplant.

The need for *ex vivo* analyses or body fluid analyses to predict things like drug sensitivity and treatment response in the area of cancer therapy. Serum methods for measuring the degree of tumor-cell kill. A general concept which I put together was a need for a broad repository and access to human tissue samples for research.

[Richard Bold]
Using Bob Cardiff's experiences as a pathologist and his experiences in terms of mouse models, in particular breast models, we would put forth a project that aims to establish a computerized repository to allow better pathologic description of tumors that incorporates real-time analysis, using 3-dimensional imaging and mathematical algorithms to eliminate the subjective analysis of tumors and allow a more objective analysis of tumors. This should incorporate some of the molecular proteonomic and metabolomic descriptors for that, and correlates with a database that brings forth the histological progression of tumors and the

clinical profile. This would just establish a database for pathology, using, initially, the mouse model of breast cancer because of its availability, but then use that as a foundation for adaptation to human tumors.

The second project would incorporate some clinical aspects in terms of treatment, treatment response, and individualization of tumors. The approach would be best characterized as identification of surrogate markers, given the limitations on obtaining human tumor specimens in a serial fashion. We picked a few that Angela Davies and Phil Mack have had experience with in terms of hypoxia, angiogenesis, and inflammation. Serum surrogate markers that incorporate both the host and the tumor, initially and in response to therapy, which could be used to predict both tumor response and host toxicity. We discussed using technologies such as NMR or Luminex with immediate real-time feedback. That pulled together the experiences of Davies, Mack, available technology (micro-NMR for small-sample analysis, Luminex technology), and Charlene Schaldach for modeling data for prediction of response.

[Ralph de Vere White]
We tried to be focused. Davies and Mach said that if they had to pick simple protein targets, they would look at PIE-1, VEG-F, and Osteopontin as specific markers to assay. Bob Cartiff said that tumors dying in response to treatment should demonstrate some kind of systemic inflammation. How about cytokines? We developed the cytokine/angiogenesis themes.

Which of these tells you that your bone marrow is going to go down? We are looking at quick predictors. Presumably, a predictor predicts a white cell count drop. Is there a predictor that tells us you are going to have toxicity before the toxicity occurs?

[Phil Mack]
I would say part of the goal would be to identify new markers. The markers that Rick Bold mentioned we have already explored. The issue is how do we find new markers, new targets, without having to do massive arrays and search through tens of thousands of different possibilities. This is a compromise between the things we know about and the things we need to discover. So, no, we do not have specific answers for what to look at.

[Andy Wyrobek]
The issue of discovery was raised, and how much we can invest in it. A large cost is not easily affordable with NIH funding so we left it as a question. Could not we invest early in discovery to identify specific biomarkers in cases where we know there is bone marrow toxicity and in case where we know there is not bone marrow toxicity, and thereby identify candidate genes and then build more high throughput assays that we could use. This is the alternative to just taking a guess at what it might be and testing those guesses.

[Ralph Green]
It is not likely to be a single marker. It is all about pharmacogenomics, essentially, so that you might be able to identify, depending on the agent, who the people are that might be susceptible to untoward effects that would cause unknown failure.

There is tremendous need for discovery, and discovery can be in the context of a specific tumor; it could be the context of a specific organ and less toxicity for example. This is why the issue develops in radiotherapy of bone marrow toxicity or gut lining toxicity. We could model that in a human experiment, and look in the serum at the specific place where we radiate the

body to kill specific cells. See if we can see in the serum or in the urine the damage caused in those cells.

[Sally DeNardo]
I think this is a very important area. Only a few people have developed information to build on and develop a proof of principle in looking at therapy.

[Rick Bold]
Ralph de Vere White asked, Are we ready to say we are excited about what we propose? Phil Mack, in your next Phase I or Phase II trial do you want to do a subset analysis, grabbing plasma, doing Luminex on 15 cytokines that you pick? Some discovery; some known, and have the data for follow-up and say this is really what I want to do because I want to take it to the next step.

Group 2

[David Gandara]
There are three proposals from our group. The first was a proposal for using proteomics to determine risk of recurrence of superficial bladder cancer.

The second, which I will defer to the investigators, is developing a virtual cancer cell model based on a computational model to exploit the malignant cells.

[Ken Turteltaub]
We also discussed many different technologies and really focused on what we felt the needs for these are. Proteomics for bladder cancer involves using a variety of different technologies. We are thinking discovery ranging from the use of mass spectrometry to the use of transcript analysis in a variety of array types to 2-D gel electrophoresis for proteins in a DMR for metabolites, and other sorts of analyses. We would work to find markers that are useful particularly for bladder cancer, but also to define the risk of recurrence and the risk of cancers likely to proceed to malignancy.

The virtual cancer-cell model is a highly computational approach with the goal there of actually utilizing phenotypes and trying to predict, if you know something about the metabolic pathways, which components are likely to change and how they are going to change. If you were good at doing that you could start to look at how therapeutics would affect tumors as well as prognosis.

We wanted to think way, way out there since it requires a lot of understanding of individual pathways, how things interact, how things get shunted, and having the right models in place to do this accurately. It has to be strongly coupled with experiments to validate the approaches.

The third topic we discussed was what we entitled Selectivity of Contrast Agents.

[David Gandara]
We were intrigued by the bio-briefcase, and particularly the potential of translating use of aerosol monitoring for cancer purposes. One proposal was to be able to use this in lung cancer wherein the patient coughs up material for screening cytology. Sputum cytology has

not been very effective for this purpose; the filter would catch cells and then we would be able to phenotype them.

The second proposal was to use the concept to look at individual variability and drug metabolism. We would look for an intermediate metabolite or whatever of the drug with this aerosol method after a single administration to see how that patient was going to handle the it. The concept here would be the same as the erythromycin breath test but only taking it to a higher and more sophisticated level. That i test is commonly used in experimental purposes to determine liver function, for example.

[Ken Turteltaub]
That is a technique that could be useful with instrumentation like accelerator mass spectrometry, NMR, and other forms of mass spectrometry, particularly for the drug-metabolism component of this.

The third piece here we called Diagnostic Fingerprinting, and the idea there would be to look for some ways to address susceptibility, mostly based on genetic fingerprints. So, we are talking about looking for specific things there such as SNPS, haplotypes, or STR changes. Part of that would involve having to work on cheap genome sequencing of individual people to find targets or mutations that looked interesting. A variety of things could be envisioned including single-cell sequencing. The ultimate goal, as I mentioned, is dealing with susceptibility, if one could find markers that would be useful in certain patients for whom you could predict outcome.

This gets back to a high throughput, discovery process to find signatures, combinations of molecular markers that could be useful in diagnosing, treating, and following up of cancer. The focus here is on the pre-malignancy phase. This would involve a lot of different technologies, many of which were discussed today.

[David Gandara]
So, for example, in cancers where there is a pre-malignant or a carcinoma *in situ* phase, this could be applied in high-risk population, patients with dysplasia, for example, to determine which patients are likely to develop a malignancy.

[Ken Turteltaub]
The first component of this was really thinking about doing this in clinical populations where we would have patients that David and others have, combining that with the analytical things that are happening at Livermore now as well as at Davis.

[David Gandara]
So, the second one was a proposal by Kent Lloyd to use our resource in mouse modeling to do what was referred to as a genome-wide screening.

Ken Turteltaub
What Kent Lloyd is thinking about, since there's lots of mouse strains and modified mouse strains that he has, particularly ones for which he has embryonic stem cells, that we could produce mice from, and the idea would be to modify mice or to make mutants. Altered mice would be used to study stages where you can take specific genes or components of the genome, and either replace them or do other things to them and study these models by

analyzing plasma and other things to look for changes. This could be useful ultimately in screening and looking at changes that occur when you have a disease or when you have specific types of mutants.

[David Gandara]
While some of these proposals are broad-brush strokes, the next is very much focused. It was proposed by Allan Chen, a UC Davis radiation oncologist who works on the interactions of topoisomerase agents, chemotherapeutic agents, and radiation. His work on DNA and proteins might be furthered by looking at the pharmacokinetics of Si hybrid inhibition of DNA.

[Ken Turteltaub]
There were also some suggestions about using laser imaging and combining those with therapeutic approaches. If you could use lasers as Stavros Demos is doing to find contrast agents or other agents to sensitize tissues to locate cancers, and either ablate them on the spot or photosensitize them.

[Jim Boggan]
If you do not have useful signatures on-hand, it requires a lot of fishing now to find them. We looked at the possibility of using urine, blood, and sputum for finding signatures.

[Cheryl London]
A serum-based signature has been developed for ovarian cancer that is fairly accurate. You might want to start with that to see if you can replicate that in an aerosolized sample.

[Dennis Matthews]
You are saying aerosolize the sample as opposed to breathing out the sample?

[Cheryl London]
See if you can recognize that pattern in something that is aerosolized or exhaled rather than in a serum since there is already a known correlative signature.

[Unidentified speaker]
I'm involved with the BAMS [biodefense aerosol monitoring system] project. You asked how close are we to using our technology for this. There are 20 of us on the team and this is something that we discuss all of the time. It is straight out of biodefense. The main goal is how many particles can you get through as fast as possible with the least number of false positives. On the horizon is this sort of medical application. We have already worked on taking sputum samples, putting them in a nebulizer, mixing them with 50 ml of distilled water, and running them through a dryer. We see particles. We can actually detect virulent versus avirulent viruses in tuberculosis spores and we are writing up a paper right now showing those results, seeing different signatures, just on those two spore types. We are very interested in using this BAMS system in medicine.

[Kodumudi Venkateswaran (Venkat)]
We know the signatures we are looking for. Primarily, we know we are looking for the agents of interest and that is why the assays were developed. Again, for us, the BAM system is probably the closest we have because we are looking for a broad spectrum of things. But any of the Luminex technologies can be programmed if you know the signatures clearly; otherwise, it might be kind of hard.

Group 3

[Kit Lam]
John Knezovich and I met in this group and we have expertise in a lot of NMR spectroscopy, mass spectrometry, combinatorial chemistry, and immunotherapeutics. We talked about NMR methodologies and the new magic angle-based approach. We have experts in AMS here, applications in molecular kinetics, and in LCMS. We talked about how we can use it to do molecular profiling.

I talked about combinatorial chemistry, how we can apply it to therapeutics as well as proteomics, and using chemical micro-arrays in conjunction with mass spectrometry.

We talked about molecular modeling and how we could enhance combinatorial chemistry to develop drugs for therapy.

Also we had an expert in the group about DNA-detection techniques and how we can apply it for cancer detection and infectious agent detection using existing techniques and some new techniques that can be developed.

We also discussed instrumented cells (how the cell is trapped in wells) and perhaps we could use some this to do cellular studies.

Use of the multiplex PCR and multi-peaks was also discussed.

<u>Proposed collaborative projects</u>

One of them is the detection by DNA methods. We talked about chips and multiplex methods. Another is early detection of sources of infection in cancer patients. Cancer doctors commonly see patients who are totally immunocompromised because of chemotherapy or because of bone marrow transplantation. The white count is almost zero, they get infected, but we do not know what the source of infection is. One common source of infection is fungal infection. It is hard to culture those. Hard to make a diagnosis. Usually it is late when we make a diagnosis. It would be great if we could have a detection kit where we look for some infectious agent. The patient gets a fever. Let's do some tests and find out whether it is a fungal infection and what kind it is so we know how to treat it. It is not directly related to the cancer itself but the infections are complications of cancer patients. Clinically, if we can do something like that, it would be useful.

The second project is the detection of cancer genes in serum and body fluids for early detection of cancer. Paul Gumerlock and Elizabeth Vitalis, and several others already are working on that. We discussed detection of infectious agents that can cause cancer including HPV and Helicobacter pylori among others. Maybe you can use biodefense techniques to look for infectious agents that are related to cancer, and are either causing complications of cancer or causing the cancer itself.

The next project we discussed is about applying Monique Cosman's expertise and how that can be used to understand ligand receptor interactions. We had a lot of discussion about how to use fluorinine and also N^{15}. We can label the protein and understand how we can study the

interaction, the structure, and so on. One of the limiting steps we discussed is that we do not have high throughput methods at Lawrence Livermore National Laboratory. Right now they do it manually, one at a time, so it takes a lot of time to do the analysis. If we had a wish list, it would include a machine to do autosampling. Then we can increase from doing one sample a day to 30 a day. It costs about $50K.

The other wish is a cryoprobe that can really greatly increase the sensitivity of the detection, but at $300K it is expensive. I think NMR technique is important. We can look for ligand-protein interactions and so on.

Another project is in proteomics. They have the mass spec techniques using FTMS, tryptic digest, and bioinformatics to analyze a whole host of fragments for peptides that derive from proteins in host serum. We are interested in not only serum profiling, but also using ligands to identify proteins that bind to those ligands. Can we use mass spec to tell us what protein actually is attached to those ligands? Then we can profile it with the thousands of ligands. We have thousands of proteins and we can match them all together.

The other project is SIMS TOF to analyze intracellular protein kinase PET imaging agents to find out about localization of those imaging agents, and whether they phosphorylate inside the cell, where they are, and so on. That may be helpful. Regarding this project, Gerry DeNardo brought up an important point that instead of using only a rational drug approach, the discovery approach (molecular modeling), or using the irrational approach, combinatorial chemistry, we should use both of them together.

The last one we talked about is metabolomics that Monique Cosman discussed. She is working with Angela Davies at UC Davis about a metabolomics plan to look for buccal mucosal cells and using LCMS and NMR techniques to look for some unique pattern and drug metabolisms.

[John Knezovich]
With regard to looking at such valuable approaches such as metabolomics, which integrates all the metabolic processes in an organism, part of this discussion really was more reductionist in relating things like the instrumented cell to technologies that are cutting-edge in developing nano-SIMS, AMS at the single-cell level. Technologies that give us both molecular identification, quantitation, and localization within the cells, are at the forefront of understanding basic fundamental biology in single cells. We are struggling with how to best package that in the cancer context, but I think that is part of the discussion that is emerging.

[Dennis Matthews]
I wanted to add some suggestions. One can use the Midas technology. The fiber optic with multiple beads on the end of it that can be functionalized, and we thought it would be a great idea. Ralph de Vere White, in fact, has considered using tissue probes inside the bladder. You could look for p53, or you could look for other enzymes.

[Sally DeNardo]
You could use the sensors of your choice, including any of the binding agents including panels of peptides, antibodies, SHALs, or sensors for things like p53 or other molecular markers of not only malignancy but also information that might allow staging. Thus, while you might not determine depth directly, you might know the depth through its molecular characteristics.

[Dennis Matthews]
I think you also want to use similar technologies to detect SHALs.

[Sally DeNardo]
Then of course, we might link them with sensors with potential for follow-up therapeutic surgery or other procedures.

[Dennis Matthews]
I'd like to ask a question of John. Does it make sense to use AMS at the single-cell level?

[John Kenzovich]
If you want to look at something as fundamental as cell division and at where the carbon of an individual cell goes as it is dividing, it is a beautiful tool. John Vogel, do you want to make some other comments?

[John Vogel]
We have already started working on this using yeast cells, growing them so they have a fairly large component of C^{14} (one in a hundred to one in a thousand labeled carbon atoms) and then we take a single cell and drop it into a pot of other cells and we can separate it out. You push it through an LC and when you get a peak on your LC, quantify it by AMS where that C^{14} will only come from the one cell, and so. We have started doing the preliminary tests on this. However, it is a purely biological tool because you have to grow the cells already labeled; it cannot be added later.

[John Knezovich]
Right. By putting a labeled cell in a pool of unlabeled cells, you can do molecular identifications, using LCMS and other techniques that AMS does not do. Nevertheless, we can still quantify metabolites because they all came from one cell. The C^{14} is in only one cell.

Group 4

[Fred Milanovich]
I saw many energetic people at UC Davis. I saw access to clinical samples and clinical trials. We can't overlook that access while we develop a priority list as we go forward. I tried to think about those things at Livermore that are cutting-edge or thereabouts but are not at the invention stage. I think it is very dangerous that a lot of these require invention upon invention -- we really should not start there. We should start with something that is unique that we are doing well and apply it to a problem in a new way. For example, we have a 150 bead-set protein array that we could get into a clinical trial as quickly as that trial can get set up. We have a brand-new mass spectrometer that can do on-the-fly measurements of biologicals and nobody else has that. We ought to think about how that could link to something. Aerosols are an excellent discovery tool even though "discovery" to some people is a bad word. That is where major inventions have come from, from discovery.

We felt that some consideration should be given to supporting the development of pilot data, because, if we are thinking NIH, we almost have to have the problem solved before you are going to get them to listen to you.

[Paul Gumerlock]
Moving on to hot topics, number one is a prostate cancer study looking at Ca^{41} to measure bone loss and maybe equate that with metastases. It also has a nutritional aspect that Jim Felton obviously is very interested in. The plan would be to feed Ca^{41} to newly diagnosed patients who are having prostate cancer surgery, and then comparing the release of Ca^{41} with recurrence to see if it actually is more sensitive than PSA for tracking recurrent disease. The Ca^{41}, once it is taken in a dose, is in the bone for up to 5 years and you can continually measure it in urine over a very long period. It could measure bone loss and may in fact be related to patients who are more likely to get fractures.

We have started to write the proposal for bio-aerosol MS. We're going to look at what comes out of the breath of a lung-cancer patient, prior to them having surgery, and then we will have a control by testing that patient after the tumor is taken out. In addition, we will look at the sputum from the patients before and after surgery, and we'll have the tumor tissue to then look for the particular markers within the tumor itself.

The cost was estimated to be about a thousand dollars per sample, and whether it will be quantitative was questionable. We certainly want to do with this protein, the first 150, and then additional proteins or metabolites as they come on and may be of interest.

The other thing that we really want to do is develop a panel of proteins where we have quantitative ratio of phosphoralytion to total. This is going to be very key for looking at activated pathways in individual patients before treatment and then seeing how treatment and its outcome are related to modulation of the phosphorylated-to-total ratio. Are we shutting down both sides of the EGFR pathway or is it just one side and the other is still on, and what that means? We thought this was a very important assay to take the time to develop as a foundation for many pathway studies in the future.

It was pointed out is that there in fact is an RFA out now to research circulating tumor cells or circulating tumor DNA then we absolutely should respond to it. Monique Cosman will get our teams together in some way and address it with Pixie, STIM, and the mutated tumor DNA. I think focusing on the DNA assay rather than on circulating tumor cells would be a strength that would face less competition. DNA quantification is a very important issue for us. It would be a foundation for future studies, particularly in evaluating radiation-sensitizing drugs. If we are assaying down regulating DNA repair genes, we would need a platform where we quantitate differences in DNA damage or the amount of repair that occurs so that we can then utilize these assays in clinical trials to see if therapies make a difference in the level of DNA damage.

To echo Fred Milanovich, preliminary data is needed if we are going to be successful in getting grants.

Some of the discussions might relate to the premalignant lesions that we heard from Group 2. Fu-ton Liu focuses on melanoma and atypical nevus in patients. This presents a very accessible series of events that lead from what is likely to be premalignant lesions through malignant lesions where we can do sampling. This would be a long study looking at different sizes, and how their molecular profiles or proteins vary. Importantly, it was noted that BRAF is one of the mutations that is quite often found in melanoma, and we've just had an Letter of Intent approved for clinical trial of a BRAF inhibitor at UC Davis. That allows us access to the

drug for preclinical studies. The methylated p16 has shed DNA that also fits into some of our other research.

We discussed trying to use imaging to develop optical signatures of cancer. Because tumor images are easily accessed, perhaps imaging would be very valuable. This might develop along the lines of Stavros Demos' use of imaging to identify superficial bladder cancer.

It was noted that quantitative RT-PCR is solidly established at Lawrence Livermore. We need to take advantage of that if we are looking for changes in lesions.

The one clinical issue that is very important is that we have to be able to detect less than 10^9 cells. This is the point where almost every cancer is first clinically detected so we've got to beat this number or we are not doing our job correctly.

Minimal tissue, maximum use; how do we use very sensitive techniques?

Regarding technologies available at Lawrence Livermore, the computers, nanotech stuff, platforms that exist, and Liquid Chromatograph Mass Spectrometry.

If you are going to run samples through every one of these to get as much data as possible, it would cost about $12,000 per data point and with thousands of data points a million dollars is gone in a flash. We really need at this point to generate data.

What is in our future? I think it is adding markers to beads.

One of the main topics in our session was galectins. This is a brand-new class of proteins that seem to behave irrationally in the presence of cancer. Was it up or down regulated? That is when we got the technique solidly in place. Venkat can make an assay for these compounds, and you in therapeutics want to do the discovery. That would be a really nice marriage there.

Now it is pretty well confirmed that certain infectious diseases lead to cancer. We're studying infectious disease and ramping up in that. We ought to try to find somebody in the two organizations that is interested in that area, and we might be able to grow something out of that.

[Jim Felton]
I think that is a real interesting point. I think one of the reasons I'm interested is if you look at chemicals and oxidative damage from infection, you always get a synergy. And, what do we have? We have the one case of induced aflatoxin liver cancer and hepatitis, but I think that other chemicals and infectious diseases have not been studied, whether eating heterocyclicamines or eating PAHs, and you have quite a bit of gastric infection from pylori. It has never been looked at together and I think this is really worth doing, especially with having the ability to diagnose exact strains.

[Cheryl London]
With regard to mutation analysis and identification in the serum, we have a large number of patients we've been following with kit ITDs that do or do not have a tumor burden, and it would be very easy to look in the serum because the ITD is the mutation, the signature. So that would very rapidly provide some preliminary data on techniques.

[Paul Gumerlock]
Do you have pre and post treatments?

[Cheryl London]
We have them, yes.

[Paul Gumerlock]
Pre and post surgeries; that would be wonderful. That is an extremely valuable resource.

[Cheryl London]
They certainly haven't done it on serum but you are welcome to try to set that up.

[Paul Gumerlock]
Oh, that would be wonderful.

Group 5

[Rod Balhorn]
We started talking about what one wants to do in terms of grants. There are potentially two kinds, the R21 and R33, where there are smaller levels of funding. But the overall goal at the end of the day for the cancer center is to bring large groups together and potentially bring in larger grants. That is an issue to think over. Tom Slezak made the point that what they are doing in bioinformatics for the protein and nucleic acid pipeline requires tremendous effort and may not be particularly amenable to a small grant. We have to think seriously about what we are putting into it and maybe there are ways of getting around it. There may be capabilities that exist.

Most of our time was spent learning about the needs of the investigators in this field. What are the kinds of things that need to be solved? One is, for example, that we need to be able to take a few cells from a biopsy or in a small region of a tissue, and be able to determine if there are specific changes present. Is a tumor present, what is the tumor's grade, how far has it progressed, and how can functional imaging reveal the functional state of the cell? Another one was to identify when cells go from being responsive to nonresponsive. So, you have cases where if you treat the cells at a particular stage, they'll be responsive. But if they have gone beyond that stage to another stage they are potentially unresponsive. In both of these areas when we talked about the technology there was a lot of overlap.

If one can develop the technology, the ideal would be, for example, an optical imaging technology that is totally noninvasive. You would like to be able to go in while you are looking at the tissue, and be able to image to get your data. I mean, that is pretty much like a tricorder (the illness scanner in Star Trek) and something that is way down the road, but that is a good point. We should seriously consider developing optical technologies.

One might combine methods. Many of you, when you think of beacons, you are thinking about these DNA molecules that come together and become fluorescent; however, beacons, as detectors of a signature, potentially allow you to set up, for example, a cocktail of SHALs that you give to someone and they bind to 10 different proteins that are the signatures of some

particular type of tumor cell or certain stage, or something like that. It could be ligands, it could be other molecules as well. So, one should consider combining techniques.

Sharon Shields and Henry Benner at Livermore are using MS in a way that could be very applicable here. There are so many proteins in the blood and you want to look for the presence of a change, or look in a tissue look for a presence of a change. It seems like a horrendous task because there are many different markers and you have to sort out what is changing, but the problem becomes significantly simpler if you look for specific markers. In the work that they are doing, they are taking proteins from the blood. They are digesting them down to fragments, and looking at the fragments. We know that there are modifications in the proteins that relate to a cancer. These can be activated forms of receptors or other molecules. If you look for a specific fragment or set of fragments then the problem becomes simpler. It is a realistic possibility for to look at things like blood or to look at imaging tissue by mass spec.

Metabolites have come up in most of the discussions. I think they are useful because they are sort of the end product of a lot of the processes. It is something that people are already demonstrating very well by taking blood or urine samples and showing that there is a tissue effect in an individual.

We discussed detecting specific mutations in genes, like the p53 gene or something like that by PCR. In reality, some of the suggestions about a DNA array would be more practical than doing PCR amplification on individual ones and probably must be more high throughput.

We often make arrays and look at RNA production or the presence of certain mutations and genes, but we can also consider making protein array or SHAL arrays, so it could be arrays of antibodies that is going to detect a bunch of signatures, SHALs, or ligands or combinations of them.

One interest was to assess the effectiveness of treatment. Is the drug working? Another question is: Once you think the cancer cells are gone, are they really gone? Can you monitor blood or check the tissue or sample metabolites in urine to determine when the tumor has come back or whether all the cancer cells (and that includes metastatic cells if it is a metastatic cancer), are gone. The simplest one that we currently know is to look at metabolites to see if there are metabolic markers because that is something you can follow noninvasively.

We discussed a case where say you want to do imaging of a brain tumor. You inject an imaging agent, intracranially, and the problem is that the agent is actually there and you can't flush it out. So, could we develop a way to actually do imaging in closed systems like that. We talked about things we already do that allow that. Energy transfer is one. You have two fluorescent molecules. One that when excited, transfers energy to the other, so you look at the emission from one. Well, they can both be in the same environment and solution, and you do not see them interacting, or actually you do not see the emission unless the two are bound and interacting. One needs to start thinking about developing reagents that become visible or become detectable only when they react because that eliminates the need to get rid of the background.

[Ralph de Vere White]

The only other question we raised was whether we can get down to doing single cells? It would be very nice to know if we can look at interactions or receptors, and can we actually know what ligands are doing, and then can we know what our blockers are doing? Can we actually look at co-repressors and co-activators? For the moment, it is all based on pretty crude measurements.

[Jim Felton (addressing Allen Christian)]
How about doing *in situ* rolling circle PCR? Could we find the exact tumor margin with p53 or something like that?

[Allen Christian]

Do you mean a tumor section?

[Jim Felton]
Either a section or through an endoscope.

[Allen Christian]
You would do something like that if you knew what the single-cell stuff was, if you knew what the marker was, you could probably use it to find it. We've done that in liver.

[Jim Felton]

Can you do rolling circle PCR without busting up the cells?

[Allen Christian]
No. It is essentially *in situ* hybridization.

[Ralph de Vere White]
If you are going to look at serum (and better to look at markers rather than cells) for molecular staging, you want to know whether the cancer is either coming back or has gone away. We talked about your rolling circle PCR. If you had your marker and then if you can get rolling circle PCR to a single cell (a single cell that has your molecular marker), two months after you've removed the breast, that person has metastases. So, it would seem to me that one of our advantages is we have the technology to do both detection of tumor cells and DNA assays and since we have both technologies, why we do not go at it in both ways because I think that would be very unique.

[Tom Slezak]
We could also think about using some of the cancer markers as an expanded definition of what normal is when we are looking at trying to differentiate pathogens exposure in the host, just perhaps a little bit further along. So, that might be an area there where we do not want to just check normal 18-year-olds from a Marine Corp base but we might also want to look at perhaps a sample of people with the five most-common cancers or something like that. So, there may be other ways to leverage the synergy between biodefense and cancer.

Group 6

[Allen Christian]

We spent a lot of time talking about the utility of single-cell research, you know, whether indeed single-cell research was worth doing and if so, what sorts of research you could properly do on single cells that you couldn't do on groups of cells. Moreover, one conclusion I think it is important to draw is that we have all sorts of interesting technology at Livermore that is not always applicable to a problem. There are some technologies or some single-cell applications that you might choose to use and in many other cases, it just does not matter. I think it is incumbent on everybody to make sure that you are using the right tool for the right purpose.

Having said that, one of the nice things about single-cell measurements is if you want to do a lot of measurements on a time course, then if you can get single cells arrayed in some sort of order you can rastorize different imaging technologies across them and always be certain of coming back to the same cell over a period of time for study. For a high throughput method, or a high throughput study, single-cell technology is very valuable.

[Hsing-Jien Kung]
You have to ask why do you need to use a single cell. If anything, the readout is a visualization. You had better have a mixture of cells. You can analyze the cell data separately and then you combine them. That makes more sense. If you use cell signaling you do not need to separate the cells and isolate them. For example, paracrine factors would be disrupted.

[Allen Christian]
There are holy grails associated with every field. We spent time talking about predictive assays for radiation therapy. A simple direct predictive assay for radiation therapy would be where you can take a cell from a biopsy from somebody and have a very good idea about how fast cells would repair and thus what kind of a dose you should give them. This is a holy grail in radiation therapy. However, It is important when you write grants that no one is going to fund shooting for the holy grail, so it is important you go for smaller steps.

We talked about pathomics a lot. It is one of those things where you get a pattern effect from the whole body where you do not necessarily know exactly what each of these things are, so you have to work your way back down. You want to find the one marker for the one disease but that has been proven extremely difficult. Pathomics is great for an incremental journey when trying to figure out how to get early diagnosis.

Although one thing that did come out of our session was that people are sufficiently intrigued by the predicted-assay notion, that we thought a series of meetings and maybe short seminars where UCD oncologists and radiation therapists come to Livermore and give a series of short seminars on some very precise problems which technology people in Livermore could then address -- a smaller version of a meeting like this

[Ralph Green]
The paradigm of looking for a panel of indicators or markers with predictive value is equally applicable diagnostically and therapeutically. It is going to really boil down to sophisticated informatics to sort out that information and then derive predictive values for whatever it is that you come up with.

[Jim Felton]

Did cell-to-cell communication come up during the single-cell discussion? It seems to me that using single-cell methods that you have developed, one can put two different kind of cells together and see what communication might do to some of the parameters.

[Allen Christian]
Yeah. We did talk about that. We cannot make two cells because if the cell divides the single cell would be too small. But, certainly if you can put two cells in there, they will be isolated for study. We are developing a slightly bigger chamber for that.

[Kit Lam]
I have an idea on single-cell interaction studies. We can first attach one single cell to an adhesion peptide-coated bead. The peptide could be the same or different. We can then grasp two cell-adhered beads with two separate micromanipulators, one bead in each micromanipulator arm. We can then use the miromanipulator to place the two cells at a predefined distance and under a predefined environment. Cross talk between the two cells can be monitored on a single cell level, morphologically with appropriate fluorescent labels or biochemically with highly sensitive analytical techniques such as AMS. These two cells could be the same or different,

While it is wonderful to be able to detect a few cancer cells in a patient, say for example five cells, with the current therapeutic regimens we have, how could we use this information clinically?

[Allen Christian]
If you have something that is so sensitive it can detect 5 cells, when would you do it. The time to apply these obviously is when you are monitoring after the initial cancer detection because why are you going to look for something if you have no idea if it is there.

[Ralph de Vere White]
If you get breast cancer these days, you have a lumpectomy and you get chemotherapy for a 2-6% chance it is going to increase your survival. If you had a test that even reduced unnecessary chemotherapy by 50% that would be a huge advance. The challenge is not to start with a diagnosis for cancer. You get a marker. You get the tumor out, and now the question is do you or do you not need adjuvant therapy. If that marker would pick up early recurrence, where we have treatments, you could treat early. I think we should aim for immediate applicability, that is, approaches that could save thousands of people unnecessary treatment. After that, we could refine it down to five cells, but I do not think that is what we should be starting.

[Allen Christian]
That was precisely the conclusion to which we arrived. This is the sort of thing you are going to apply after an initial diagnosis and therapy.

[Colleen Sweeney]
How do you define recurrence? Where do you draw the line? Is it one cell, or two cells?

[Ralph de Vere White]
We are talking about whether there is still residual cancer. To some degree, that is going to depend on markers, etc. We have an example of this in testis cancer. What we have done is

to cut back on the treatment without losing effectiveness. This is the absolute paradigm. We consider how many can we save from unnecessary or excessive treatment without losing lives and then begin to ratchet back our approach.

[Ken Turteltaub]
I just wanted to say because the issue isn't finding 1 or the 5 cells, it is knowing that something's happening, while to me it may appear to be a prevention issue, it is also a susceptibility issue. You start to watch those with increased susceptibility, and when the time is right, if it looks like something can be done or needs to be done, that is when therapeutic evaluation is undertaken. At least you know it is happening earlier than you do when you have a walk-in.

[Ralph Green]
This comments underlines and extends what Ralph de Vere White was saying in relation to the question of minimal residual disease or in trying to detect the ultimate single cell that shows cancer; we of course recognize that there are serious limitations to concluding that because a marker is there or because a cell is there that that equates with clinical cancer. The best example of that is a much-studied condition, chronic myelogenous leukemia with a signature translocation BCR-able, and the fact that it is still there after treatment doesn't necessarily signify that the patient is in relapse of the condition. Now there is evidence that there are people walking around with this marker who do not have chronic myelogenous leukemia. So, where does that leave us in terms of, you know, chasing after this holy grail of saying, if you find the marker, that is cancer. Not necessarily so.

Group 7

[James Boggan]
We concerned ourselves about whether the markers were there that we could detect and whether we needed an array or an assortment of markers that would be appropriate for assessing disease. Just because you have a cancer marker, that doesn't mean that things are going to progress whereas there are mutations that can occur that certainly indicate loss of sensitivity to disease. We want to develop the multiplex-detection techniques to blood specimens since we thought blood would be most challenging, or at least preparatory to using and looking at tissue, although you could use tissue to help define the specimens. Sputum we also thought was a challenge and expensive and difficult to do. If there are clear markers that can be looked at for certain tumors, whether it is DNA, proteins, or whole cells, we thought that that would be most amenable to the multiplex-array system. We thought about pathogens because that is where the expertise already is. Certainly, these are often present in immune-compromised patients.

We thought that there were neoplasms of choice, perhaps ovarian CA is one, perhaps prostate or bladder, if you have a marker. We thought an ideal one is lymphoma since it can lose sensitivity to chemotherapy and there are mutations that possibly can be predicted with regard to the changes in DNA or DNA fragments. Certainly, those that are secretors have a protein marker that is well characterized. You can look at it; you can pick it out; you can design your system; you can work out the bugs. As these tend to be monoclonal, the DNA is the same and it is easy.

So, our group decided they needed a second meeting; they would look at a pilot project. They could tolerate 250K to start from the NIH and maybe a little bit of seed from the group and from Livermore, and they were interested in doing that project.

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